Experimental Study on the Behavior of Fecal Contamination Indicators on Surface Water Sampled at Hanbara Intake Station

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Abstract

The behaviors of *Enterobacter* spp., *Escherichia coli*, and *Enterococcus faecalis* in surface water samples were studied. The number of coliform bacteria such as *Enterobacter* spp. and *Escherichia coli* increased in water samples from Hanbara Intake Station that is located downstream from Miyagase Reservoir. The coliform bacteria counts for samples taken from the Hayato River, which is located upstream of Miyagase Reservoir, remained constant. However, the counts of the same bacteria inoculated in saline solution (0.85%) declined. Consequently, it was concluded that coliform bacteria have growth potential in the environment prevailing at Miyagase Reservoir. Furthermore, the study suggested that the observed increase in coliform bacteria might be related to changes in quality of dammed water as well as the natural lakes. On the other hand, *E. faecalis* did not proliferate in the surface water samples obtained from the Hanbara Station and the Hayato River which were inoculated. In order to adequately assess associated fecal health risks, this study recommends that the detection of coliform bacteria in water samples be followed by the detection of *Enterococcus* spp.

Key words: surface water, coliforms, enterococci, bacterial regrowth

INTRODUCTION

The detection and enumeration of indicator organisms in a water body are of primary importance in monitoring its sanitary and bacterial characteristics. Coliform bacteria have been used as indicator organisms for assessing water quality from the perspective of fecal pollution. Yokosuka City Waterworks Bureau has periodically measured coliform bacteria in water sources in order to evaluate their safety. The Hanbara Intake Station, located downstream of Miyagase Reservoir, has been one of the water sources in Yokosuka City. Ever since the impounding of the reservoir started in October 1995, remarkable increases in coliform bacteria at Hanbara Station have been frequently

observed. Since it seemed unlikely that feces contaminated the water in the reservoir, it was suspected, among other factors, that the impounding impacted on the dam's water quality. Therefore, it was assumed that bacterial regrowth occurred in the reservoir because the water quality changed. If true, the increase in coliform bacteria in Hanbara Station samples should be reflected in the water quality of the reservoir.

Several papers have reported bacterial growth in drinking water distribution systems¹⁻⁴⁾. However, few studies have addressed the growth and behavior of coliform bacteria in surface waters. In the present study, the behavior of selected indicator organisms in Hanbara Station samples was evaluated and compared to their

behavior in different surface water samples. A prepared batch culture served as control.

MATERIALS AND METHODS

Water Samples Two different sets of monthly surface water samples were collected; the Hanbara samples, taken from the Hanbara Intake Station (St.1) and the Hayato samples, collected from Hayato River (St.2). A description of the area covered by the study is provided in Figure 1. The sample collection covered a 9-month period that ran from October 1999 to June 2000. The Hayato River is located upstream of the Miyagase Reservoir, whereas the Hanbara intake station is located downstream of it. These locations imply that the characteristics of the Hayato samples would not be affected by the reservoir, and the Hayato samples should have the same composition as the water from the Hanbara Station prior to the impounding. The calculated water retention time in the Miyagase Reservoir Miyagase was 463 days because of its total volume of about 200,000,000 m³ and an average flow rate of 5 m³/sec. The leaves and twigs in the collected samples were removed. A physiological saltwater of 0.85% saline solution served as control. All samples, the control included, were sterilized with an autoclave at 121 °C for 15 min.

Indicator organisms The *Enterobacter* spp. (*E.cloacae* or *E.amnigenus*), *Escherichia coli*, and *Enterococcus faecalis* were used as indicator organisms for assessing fecal pollution. The *Enterobacter* spp. and *E.faecalis* were respectively isolated from samples collected at the Hanbara and

Hashirimizu intake stations in Yokosuka city. The *E.coli* was derived from human feces. *Enterobacter* spp. and *Escherichia coli* were identified using the API 20E system, while *E.faecalis* was identified using the API 20 Strepto system. In addition, *Enterobacter* spp. has become a dominant species of the coliform bacteria.

Batch cultures In all experiments, batch cultures were prepared in one-liter glass bottles. After inoculation and thorough mixing, each water sample was kept in an incubator in the dark at 20 °C and enumerated at certain time intervals (Fig. 2). The batch culture experiments were divided into two parts as described below.

First, we carried out a series of comparative tests to examine the influences of the Hanbara and Hayato samples on the behavior of the indicator organisms. During the tests, the indicators in the samples were cultured for 4 days, and four trials of batch culture experiments were performed. *E.cloacae* was used as the dominant species of *Enterobacter* spp. isolated from the Hanbara station at that time.

Next, we performed a study on the behavior of the indicator organisms in the

Fig. 1 Location of Sampling Sites

Fig. 2 Schematic Cultivation Procedure

Hanbara samples. During that particular set of experiments, the indicator organisms in the water samples were cultured for a few months. Thereafter, the Hanbara samples were passed through glass membrane filter (1 μ m pore size) in order to remove suspended solids and microorganisms. *E.amnigenus* was used as the dominant species of *Enterobacter* spp. isolated from the Hanbara intake station at that moment.

Enumeration of indicator organisms

Enterobacter spp. and *E.coli* were enumerated using Standard Plate Count Agar (peptone 5 g, yeast extracts 2.5 g, glucose 10 g and agar 15 g in 1 l water). Nutrient Agar plates were incubated for 1 day at 37 °C before being counted. *E.faecalis* was enumerated using Brain Heart Infusion Agar (peptone 10 g, brain extracts 7.5 g, heart extracts 8 g, glucose 2 g, NaCl 5 g K₂HPO₄ 2.5 g and agar 15 g in 1 l water). Brain Heart Infusion Agar plates were incubated at 37 °C and counted after 1 day incubation.

RESULTS

Figs. 3, 4A, 4B, and 5 show the typical behavior of the indicator organisms in the samples, and in the 0.85% saline solution that served as control during 4 days cultivation.

The typical behaviors of *E.cloacae* in the surface water samples and control are described in Fig. 3. The indicators showed more proliferation than expected in the Hanbara samples, as indicated by the largest increase in *E.cloacae* (2.1 log₁₀ units within 4 days), whereas the indicator count for the Hayato samples remained stable. In the saline solution however, a decrease in the *E.cloacae* population was observed (2.1 log₁₀ units within 2 days).

With regard to *E.coli*, behavior patterns in the Hanbara samples were classified into two categories; regrowth and non-regrowth, as shown respectively in Fig. 4A and Fig. 4B. However, the behavior patterns of *E.coli* in the Hayato samples and the saline solutions were essentially similar to those of *E.cloacae*. The Hanbara samples displayed the regrowth of *E.coli* (2.1 and 2.8 log₁₀ units within 4 days). It appeared that *E.coli* might have regrowth potential in the Hanbara samples,

Fig. 3 Behavior of *E.cloacae* in water samples. Symbols: ○, Hanbara water sample; □, Hayato water sample; △, Control.

Fig. 4A Behavior of *E.coli* in water samples. Symbols: ○, Hanbara water sample; □, Hayato water sample; △, Control.

Fig. 4B Behavior of *E.coli* in water samples. Symbols: ○, Hanbara water sample; □, Hayato water sample; △, Control.

although they did not increase their count in those samples.

These results demonstrated a difference between the Hanbara samples and Hayato samples with regard to the ability of coliform bacteria such as *E.cloacae* and *E.coli* to grow. The difference between the two sets of samples lay in the position of their point of collection; that is downstream or upstream of the reservoir. This finding indicates the possibility of coliform bacteria such as *E.cloacae* and *E.coli* to regrow in Miyagase Reservoir. The regrowth of the coliform bacteria in the Hanbara samples might be explained by the presence of organic material in the reservoir.

On the other hand, the counts of *E.faecalis* remained constant in all experiments (Fig. 5). Compared with the behaviors of coliform bacteria such as *Enterobacter* spp. and *E.coli*, *E.faecalis* did not show growth potential in the surface water samples from Miyagase Reservoir. Since *E.faecalis* did not proliferate in the surface water samples for about 2 weeks, the detection of *Enterococcus* spp. was required in order to appropriately evaluate the health risks associated with water use.

Figs. 6, 7, and 8 show the typical behavior of the indicator organisms in the Hanbara samples after about 3 months. As described in Fig. 6, the counts of *E.amnigenus* in the Hanbara samples initially increased dramatically ($3.8 \log_{10}$ units within 7 days), then remained almost constant for about 3 months. The behavior of *E.amnigenus* in the filtrated Hanbara samples was essentially the same as that of the Hanbara samples, although the initial growth rate was slightly

weaker. Consequently, the growth factor of *E.amnigenus* in the Hanbara samples was determined to be the dissolved substances. Data showed that *E.amnigenus* counts in the saline decreased rapidly within 2 days.

The number of *E.coli* in the Hanbara samples also increased (about $2 \log_{10}$ units) for about 3 months, but their behavior in the Hanbara samples was somewhat different from that of the *E.amnigenus* (Fig. 7). For the first 7 days, the counts of *E.coli* in the Hanbara samples decreased slightly (Fig. 4B) and increased rapidly (about $2 \log_{10}$ units) between the 7th and 20th days. After 20 days, the count of *E.coli* in the Hanbara samples remained high for about 2 months at about $5 \log_{10}$ cells/ml. The most remarkable feature observed about the *E.coli* was their regrowth (about $1 \log_{10}$ unit up to 100 days) in the saline water environment. This unexpected observation could be explained by the fact that the seeding solutions used in our experiments contained both dead and viable bacteria. Viable *E.coli* might have used the organic substances generated by the dead *E.coli* for their regrowth. Similar observations were made by Bito and Matsumae. In a private communication, they reported that the *E.coli* count could increase in saline water during long-term cultivation. Another possible explanation for the regrowth of the *E.coli* in the control samples is their potential to survive in saline waters. A study by Dupray *et al.* has demonstrated that *E.coli* can survive in marine environments⁵⁾. Although, those observations are of a great importance, they need to be supported by further investigation in order for the behavior of *E.coli* in saline waters to be fully understood.

The numbers of *E.faecalis* in both the Hanbara samples and the filtrated Hanbara samples remained almost unchanged at about 1500 cells/ml for about 2 weeks after the inoculation. However in the saline solutions, the counts of the same indicator organism remained constant for just 1 week, then gradually decreased, as described in Fig.8. Moreover, Fig.8 illustrated the effect of filtration on the behavior of *E.faecalis* between the 35th and 100th day. However filtration did not have the same effect on the

Fig. 5 Behavior of *E.faecalis* in water samples.
Symbols: ○, Hanbara water sample;
□, Hayato water sample; △, Control.

behavior of *E.amnigenus* and *E.coli*, as shown in Fig. 6 and Fig. 7. Further research should evaluate the above phenomenon. It appears that *Enterococcus* spp. such as *E.faecalis* differed from coliform bacteria in

terms of their growth ability in surface waters.

Future experiments using the Hayato samples are required to properly evaluate the long-term effects on the behavior of the indicator organisms in surface waters.

DISCUSSION

Coliform bacteria such as the *Enterobacter* spp. and *E.coli* have been generally recognized as key indicator organisms in the evaluation of fecal contamination. The indicator organisms should survive in the environment but not proliferate in the water tested to give a higher than normal estimate of the cell count. However, it can be concluded from our data that *Enterobacter* spp. and *E.coli* grew in the natural water environment of the Miyagase Reservoir. The ability of *Enterobacter* spp. and *E.coli* to grow represents one of the serious disadvantages of their use as indicators in the assessment of water quality with respect to fecal pollution. In particular, *E.cloacae* is often isolated from water environments⁶⁾, and therefore it might not be so surprising that *Enterobacter* spp. can multiply in the water environments.

The findings of the present study suggest that coliform bacteria have regrowth potential in surface waters due to the influence of dams and natural lakes. Our study, however, was restricted to experimental conditions. Consequently, further investigations using the same indicator organisms but with other surface waters are needed.

Regrowth of coliform bacteria in natural water environments does in fact depend on a number of factors, such as the bioavailability of organic compounds, nutrients and indigenous microorganisms. In our experiments, organic compounds were not detected in water samples. In future studies, it might be necessary to analyze the samples for biologically available organic carbon, i.e., assimilable organic carbon (AOC) or biodegradable dissolved organic carbon (BDOC), because these organic carbons could be responsible for the bacterial regrowth potential⁷⁾.

Sathasivan et al.^{1, 2)} reported a set of data that demonstrated that phosphorus was the

Fig. 6 Behavior of *E.amnigenus* in water samples.
Symbols: ○, Hanbara water sample;
◇, Filtrated Hanbara water sample; △, Control.

Fig. 7 Behavior of *E.coli* in water samples.
Symbols: ○, Hanbara water sample;
◇, Filtrated Hanbara water sample; △, Control.

Fig. 8 Behavior of *E.faecalis* in water samples.
Symbols: ○, Hanbara water sample;
◇, Filtrated Hanbara water sample; △, Control.

limiting inorganic nutrient for bacterial regrowth in water distribution systems. In line with that finding, it might be interesting to evaluate the relationship between the regrowth of coliform bacteria and phosphorus presence in surface waters. Moreover, taking into account the fact that the water was dammed, additional research should focus on the effects of algae growth potential (AGP), chlorophyll and Nitrogen.

In a previous article, Bito *et al.* showed the regrowth of *E.coli* O157 in sterilized river water samples and non-regrowth in the non-treated samples⁹⁾. Their study suggested that biological interactions between *E.coli* O157 and the indigenous microorganisms in the river water samples might take place. The results reported by Sidhu *et al.* have indicated that indigenous microflora are the single most important factor controlling the regrowth of *Salmonella* not in water environment but in composted biosolids⁹⁾.

Streptococcus species of fecal origin has now been reclassified to the genus *Enterococcus*. The isolation and detection of *Enterococcus* is accepted as a definitive indicator of fecal pollution¹⁰⁾. The findings of the present study basically concur with previous results. Recently, the detection of enzyme β -glucosidase activities has served as the basis for the development of a defined substrate technology method for the identification of enterococci¹¹⁾. From that viewpoint, we suggest that in order to adequately assess associated fecal health risks, the detection of coliform bacteria in water samples be followed by the detection of *Enterococcus* spp.

CONCLUSIONS

It can be concluded from our findings that *Enterobacter* spp. and *E.coli* have the potential to grow in natural lakes and dammed waters. However, *Enterococcus* spp. such as *E.faecalis* cannot proliferate in similar environments. In order to adequately evaluate the health risk of a water safety related to fecal pollution, we recommend that the detection of coliform bacteria be followed by the detection of *Enterococcus* spp.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Bito, former Professor of Kanagawa Prefecture College of Nursing and Medical Technology as well as Dr. Akiyama, Assistant Professor of Kitasato University for their many helpful suggestions.

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- (Submitted 2001. 10. 29)
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